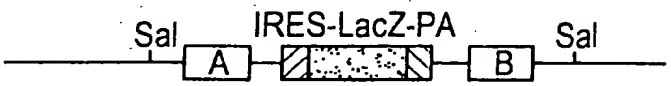
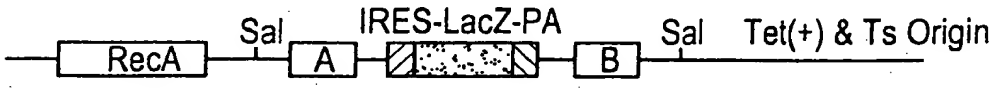
FIG. 1

I. Construction of the recA(+) and temperature sensitive shuttle vector

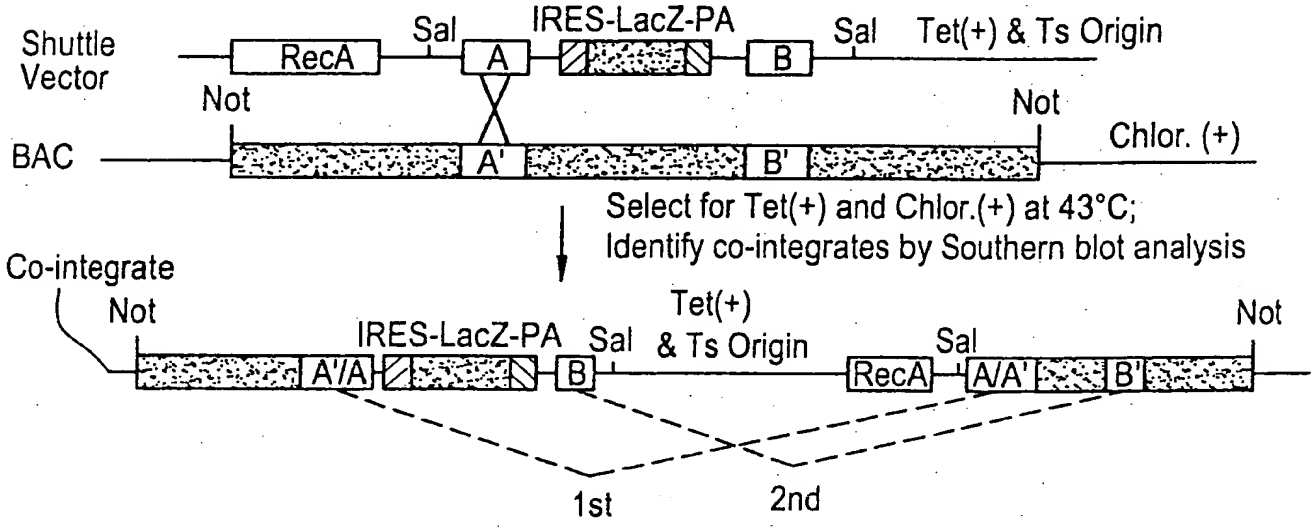
1) Clone two small genomic fragments (> 500 bp each) into the building vector (pBV1)



2) Transfer the recombination cassette into the Ts-RecA(+) shuttle vector (pSV1.RecA)



II. Transformation of the shuttle vector into the *E.Coli* host strain of BACs and selection for co-integrates



Grow Co-integrates on Chlor.plates at 43°C

III. Resolution

Restreak and select for Chlor.(+) and Tet sensitivity (Fusaric Acid) at 37°C

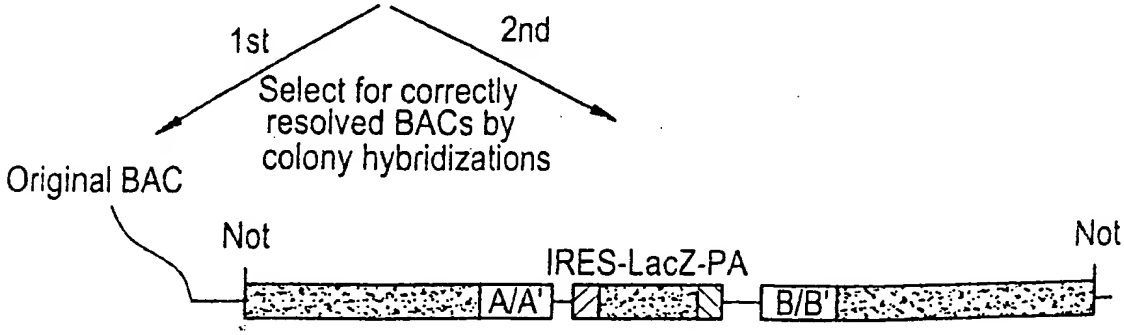


FIG. 2A

Map of the RU49 Genomic Locus within BAC169

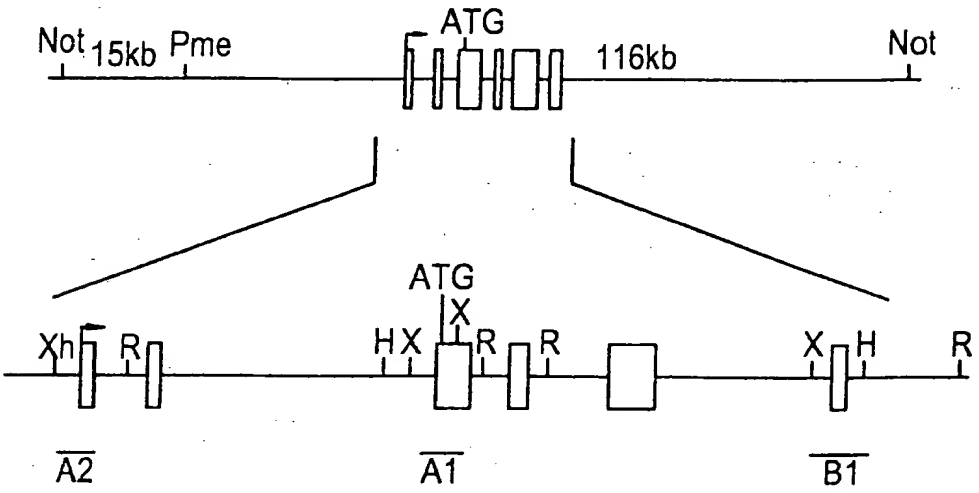


FIG. 2B

Map of the Modifiied BAC 169 with an IRES-LacZ-PolyA Insertion

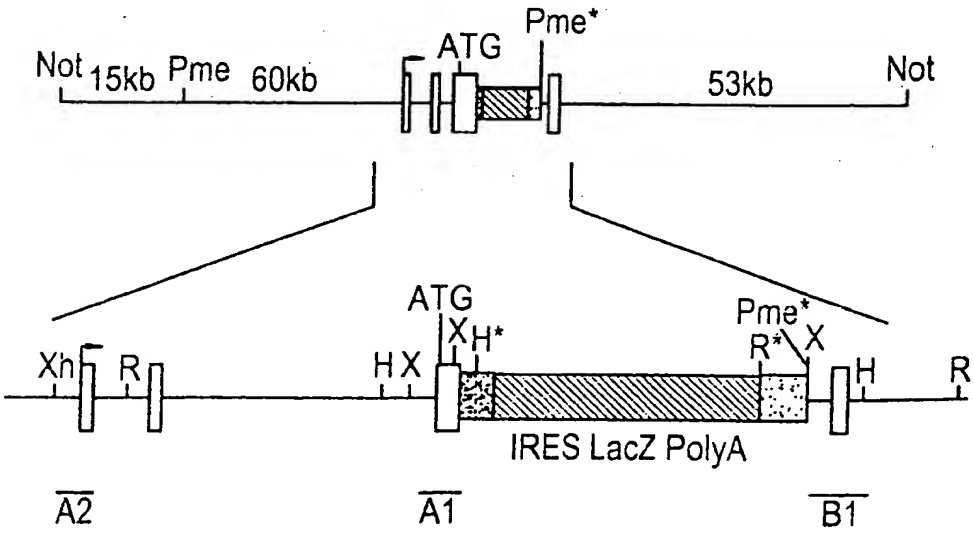


FIG.3A

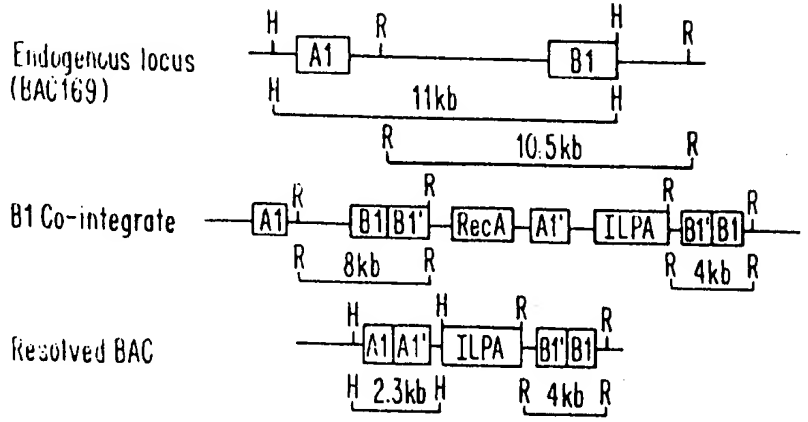


FIG.3B

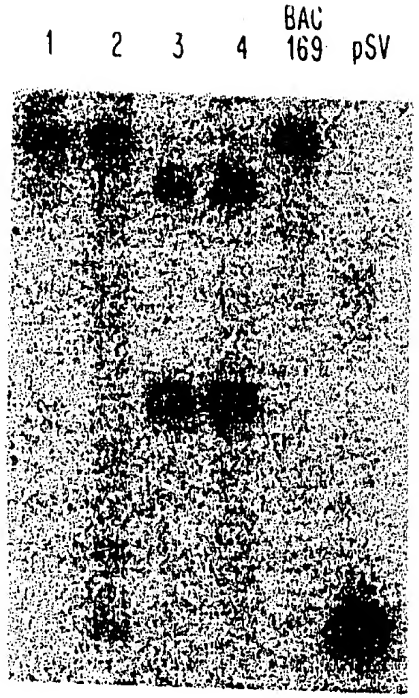


FIG.3C

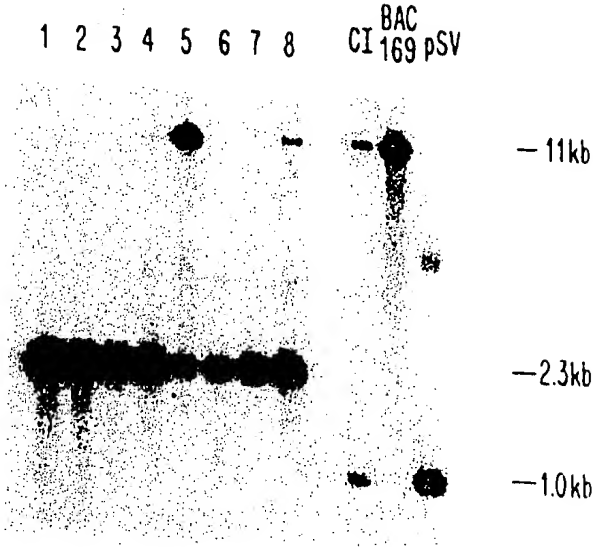


FIG.3D

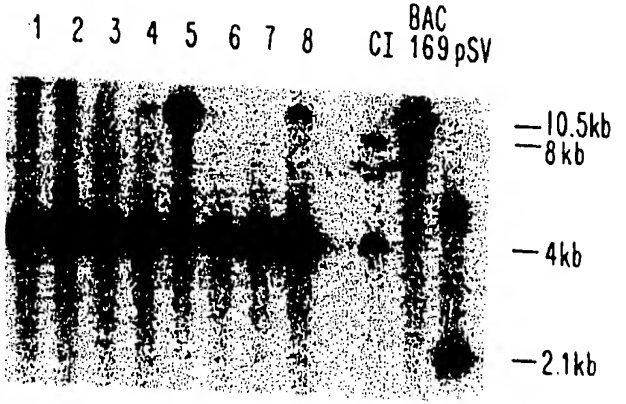


FIG.4A

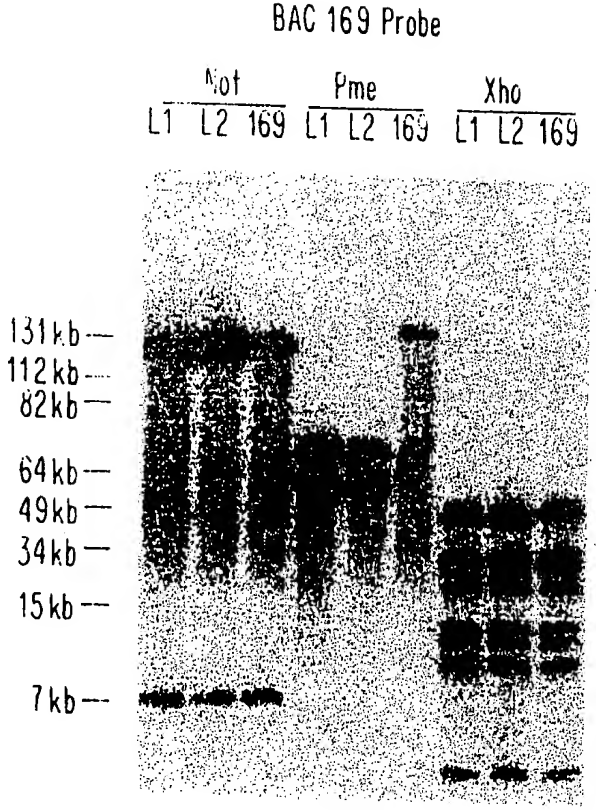


FIG.4B

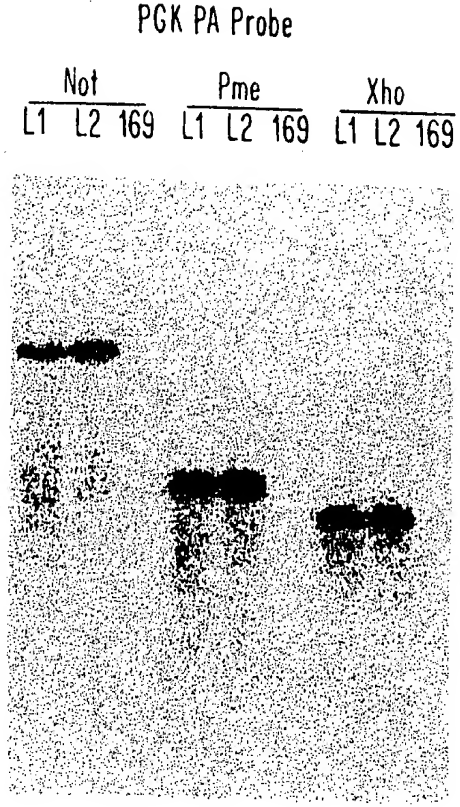


FIG.4C

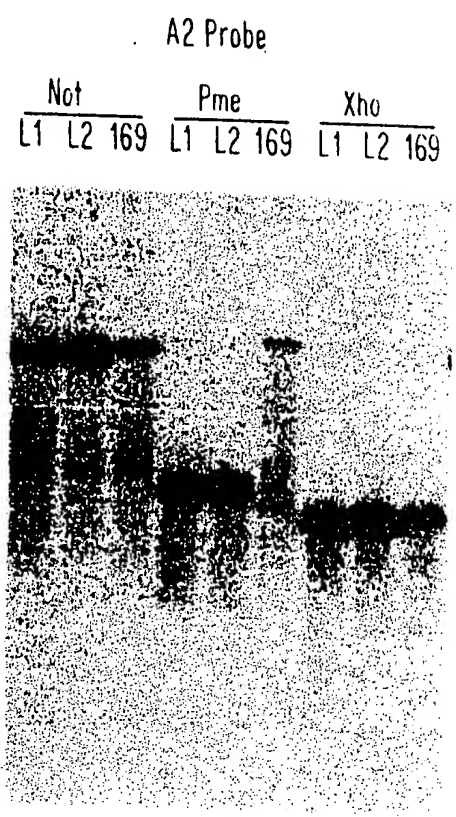


FIG.5A

FIG.5B

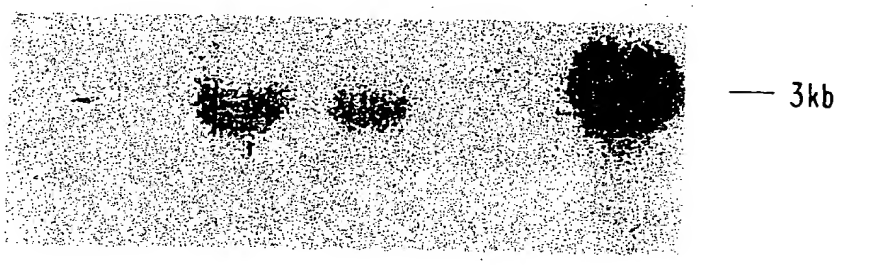


FIG.5C

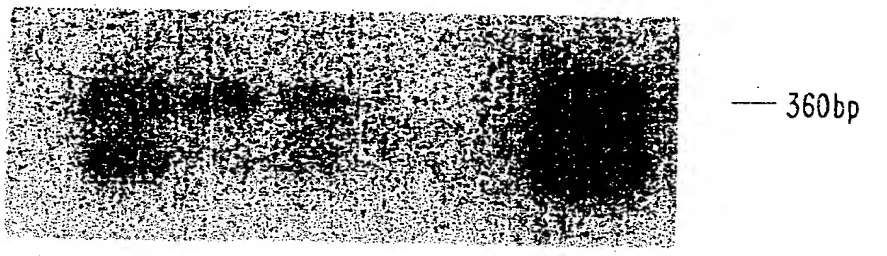


FIG.5D

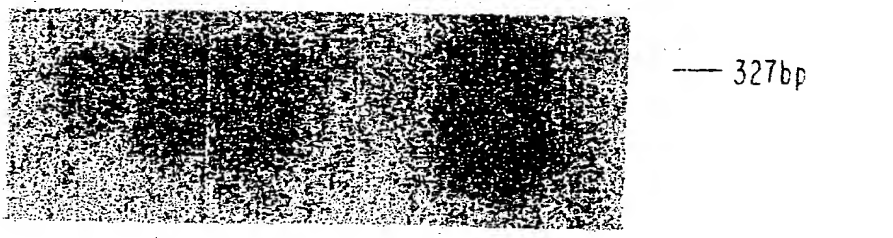
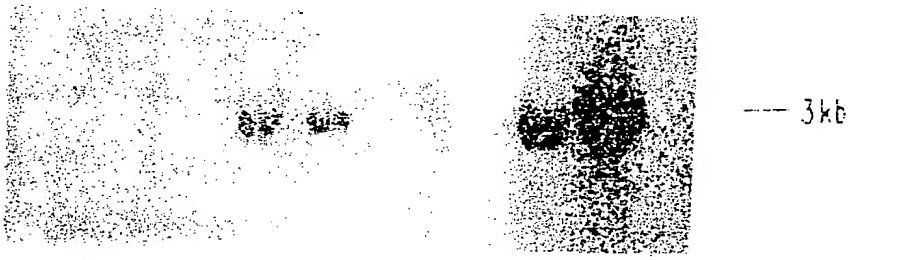


FIG.5E



08654-0-0-0-0-0-0-0

FIG.6A

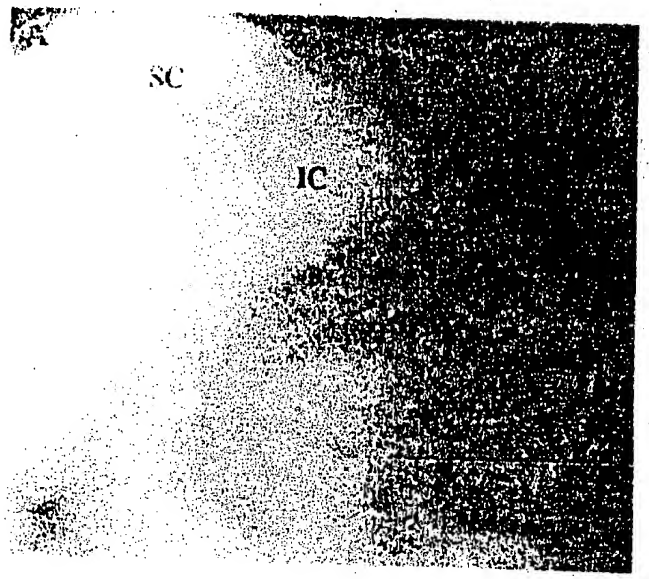


FIG.6B

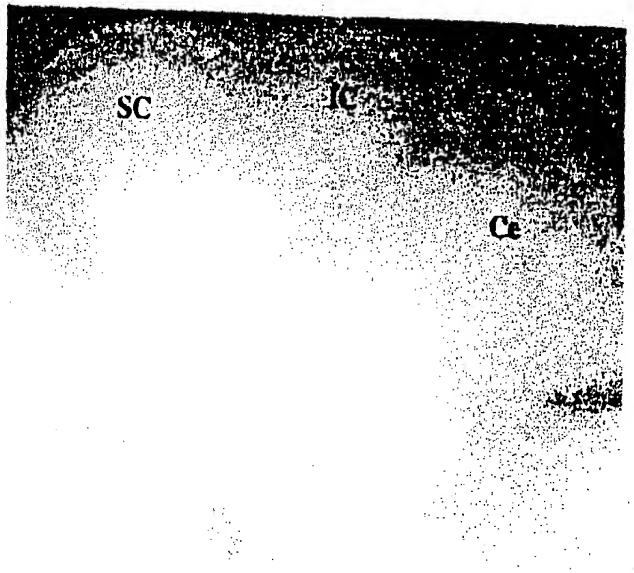


FIG.6C

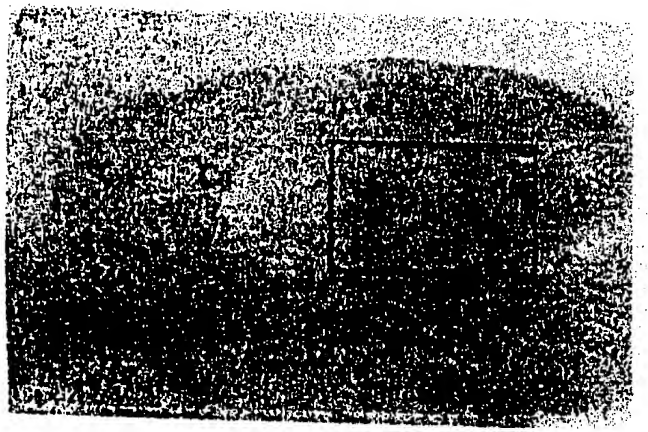


FIG.6D

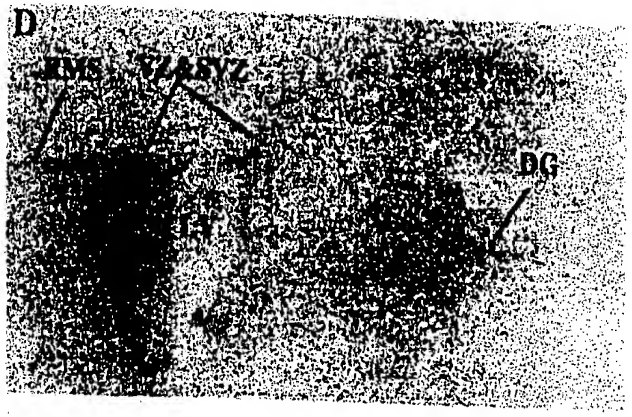


FIG. 7A

Hypothetical map of a gene of interest within a selected BAC (>150kb insert size)

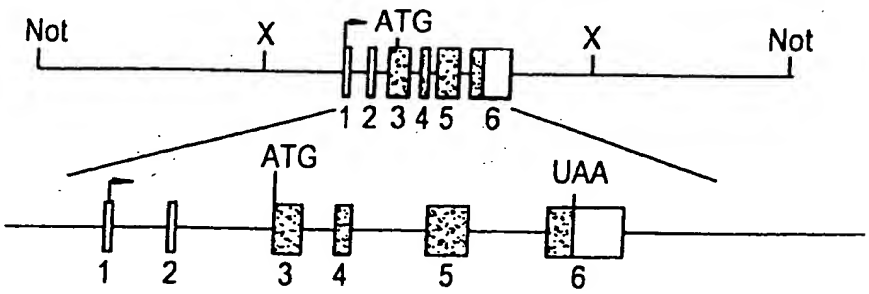
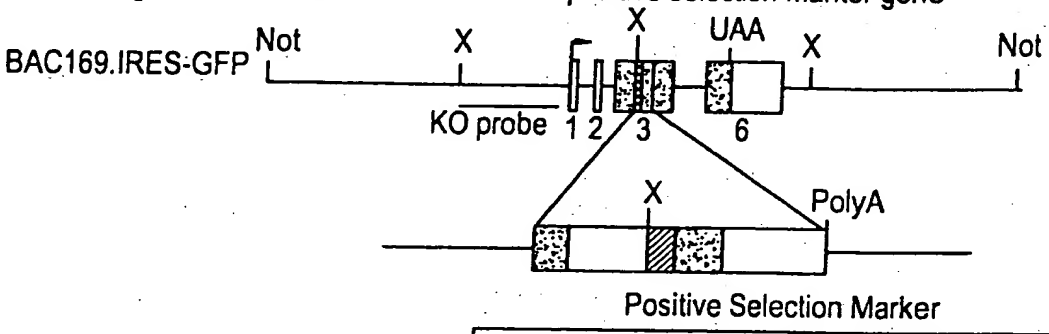


FIG. 7B

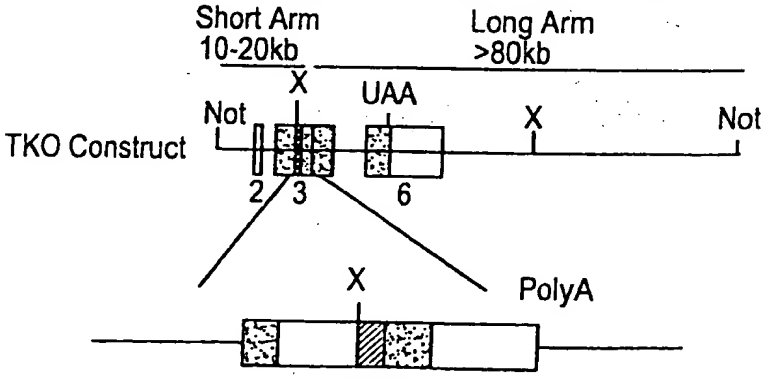
First targeted modification to introduce the positive selection marker gene



ES Cells Targeting: Marker gene=PGK-Neo-PA
Transgenic knock-out: Marker gene=IRES-EGFP-1 or IRES-LacZ

FIG. 7C

Second Modification to delete the promoter of the gene and to generate the short arm



Selection of positive clones by Southern blots:

Digest DNA with enzyme X and probed with the KO probe

Endogenous allele: >50kb

Target allele: <20kb

FIG. 8

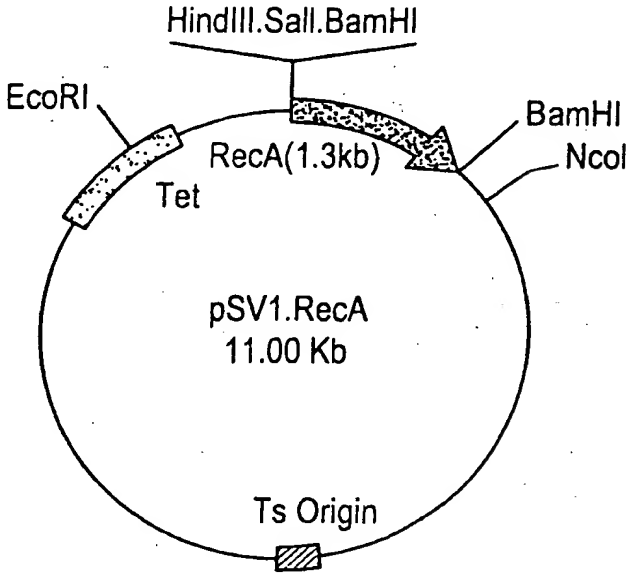

HindIII.SalI.BamHI
EcoRI
BamHI
NcoI
RecA(1.3kb)
Tet
pSV1.RecA
11.00 Kb
Ts Origin

FIG. 9

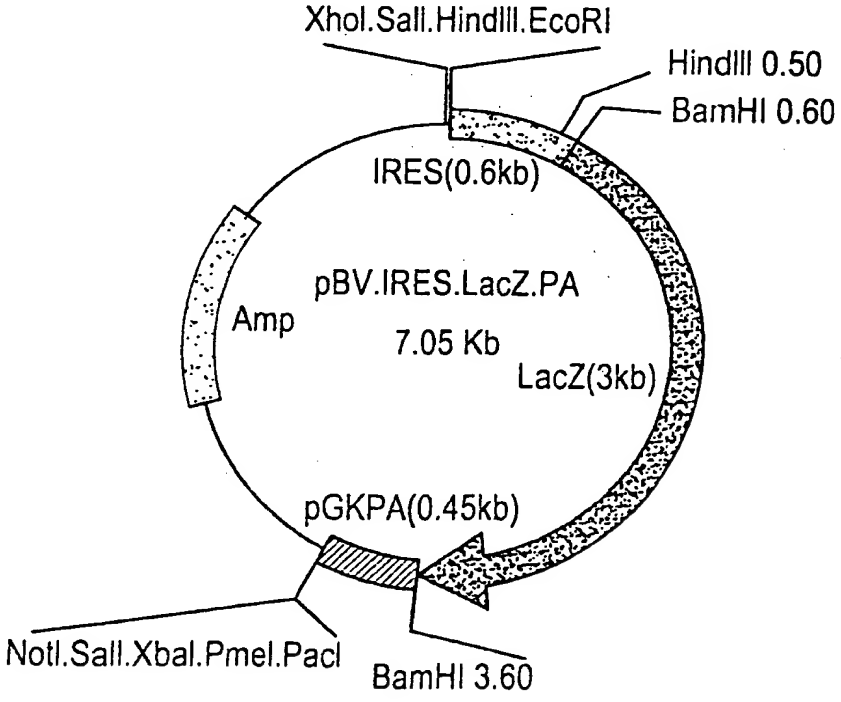

XhoI.SalI.HindIII.EcoRI
HindIII 0.50
BamHI 0.60
IRES(0.6kb)
pBV.IRES.LacZ.PA
Amp
7.05 Kb
LacZ(3kb)
pGKPA(0.45kb)
NotI.SalI.XbaI.PmeI.PacI
BamHI 3.60

FIG. 10

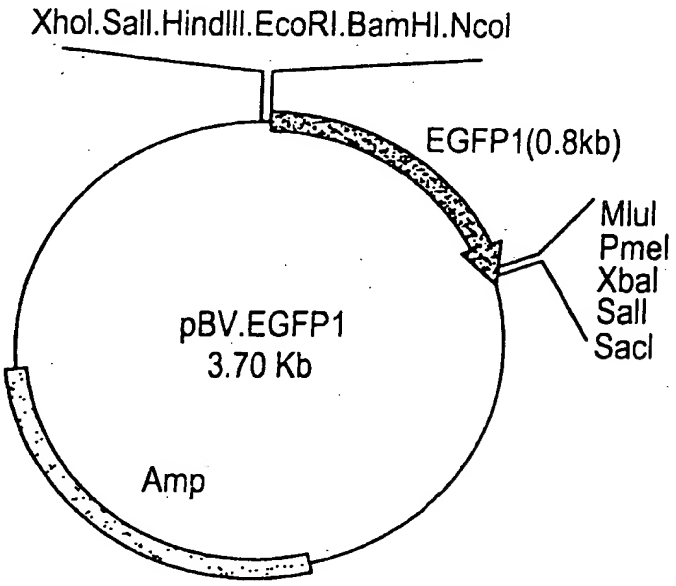

XhoI.SalI.HindIII.EcoRI.BamHI.NcoI
EGFP1(0.8kb)
MluI
PmeI
XbaI
SalI
SacI
pBV.EGFP1
3.70 Kb
Amp

FIG. 11

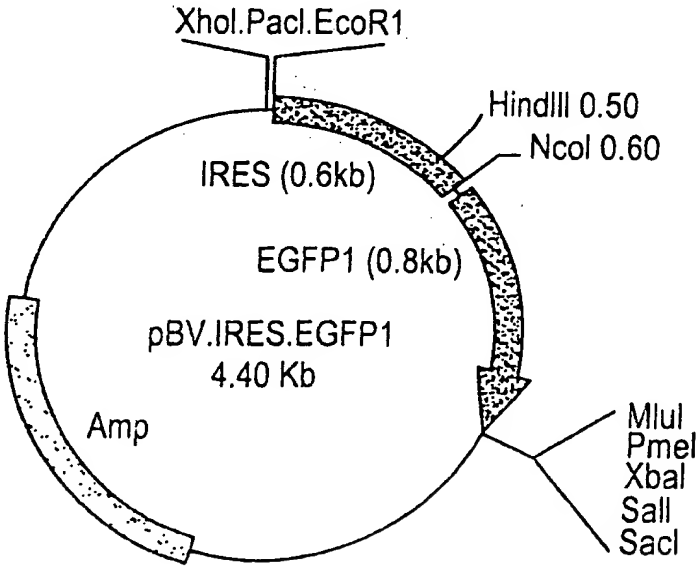
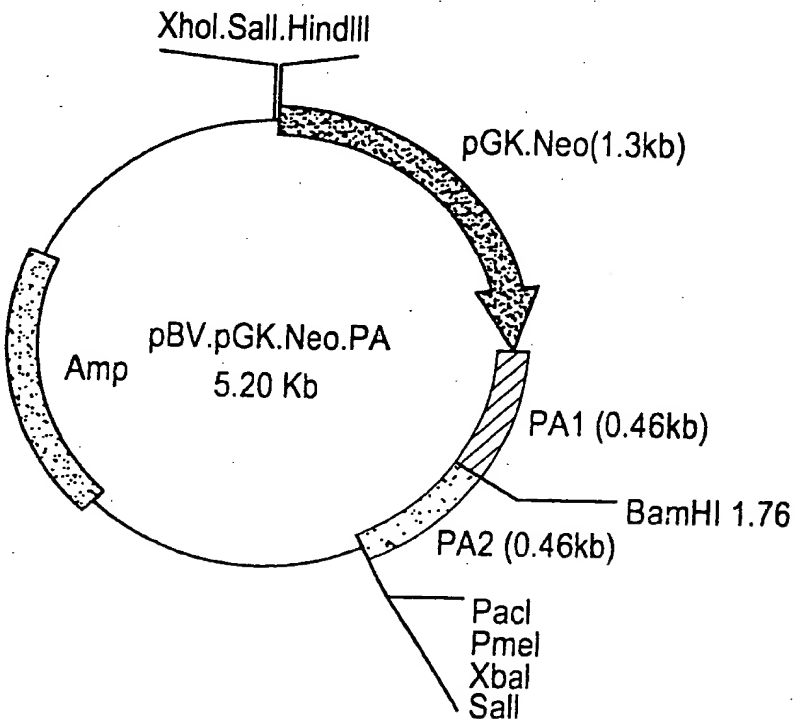
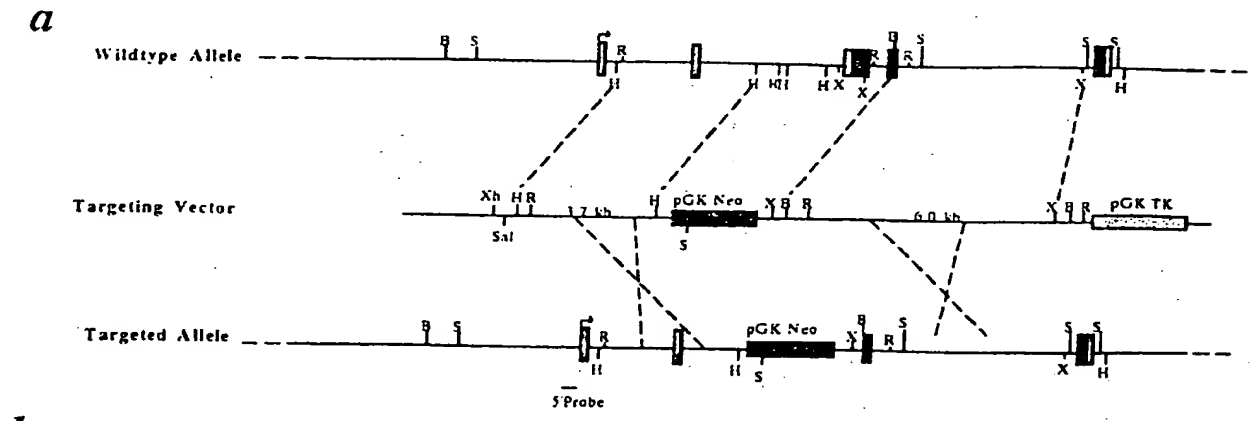
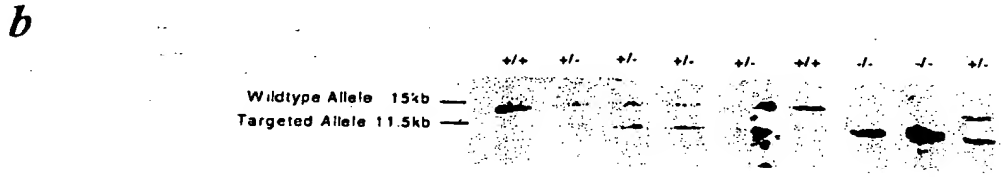
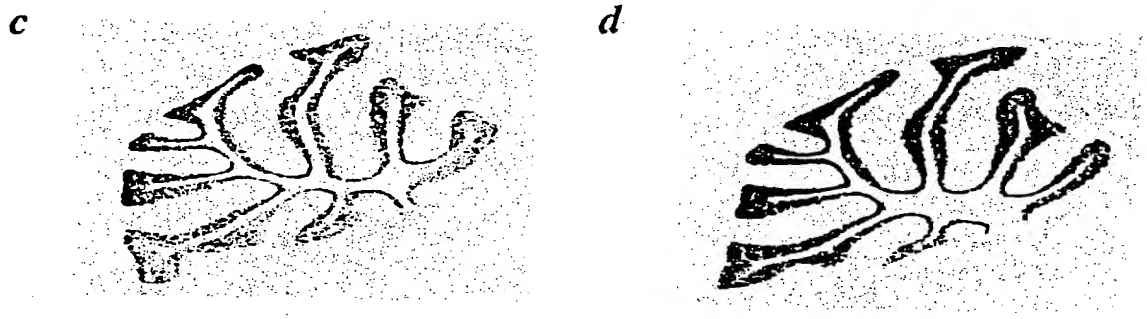

XhoI.PacI.EcoR1
HindIII 0.50
NcoI 0.60
IRES (0.6kb)
EGFP1 (0.8kb)
pBV.IRES.EGFP1
4.40 Kb
Amp
MluI
PmeI
XbaI
SalI
SacI

FIG. 12


XhoI.SalI.HindIII
pGK.Neo(1.3kb)
pBV.pGK.Neo.PA
5.20 Kb
Amp
PA1 (0.46kb)
BamHI 1.76
PA2 (0.46kb)
PacI
PmeI
XbaI
SalI


a
Wildtype Allele
Targeting Vector
Targeted Allele
pGK Neo
pGK TK
5'Probe


b
+/+ +/- +/- +/- +/- +/+ -/- -/- +/-
Wildtype Allele 15kb
Targeted Allele 11.5kb


c
d

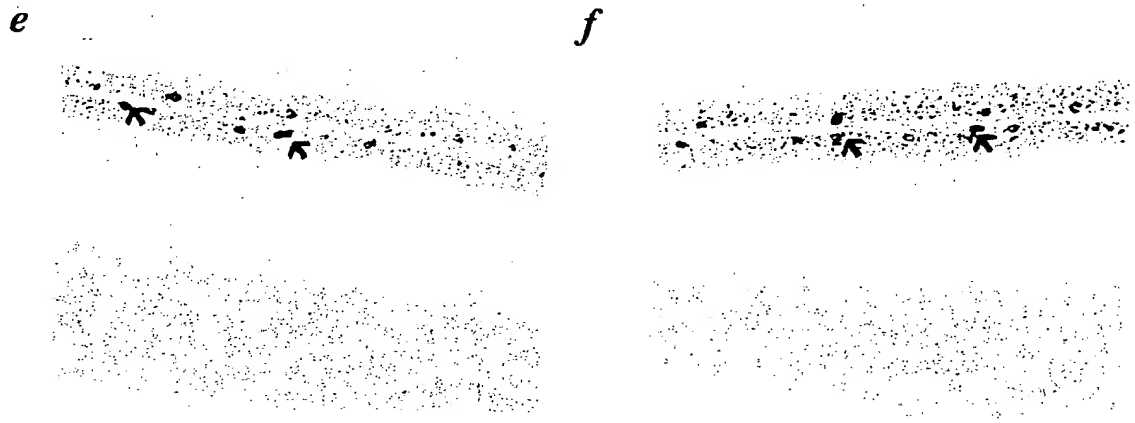

e
f

Figure 13

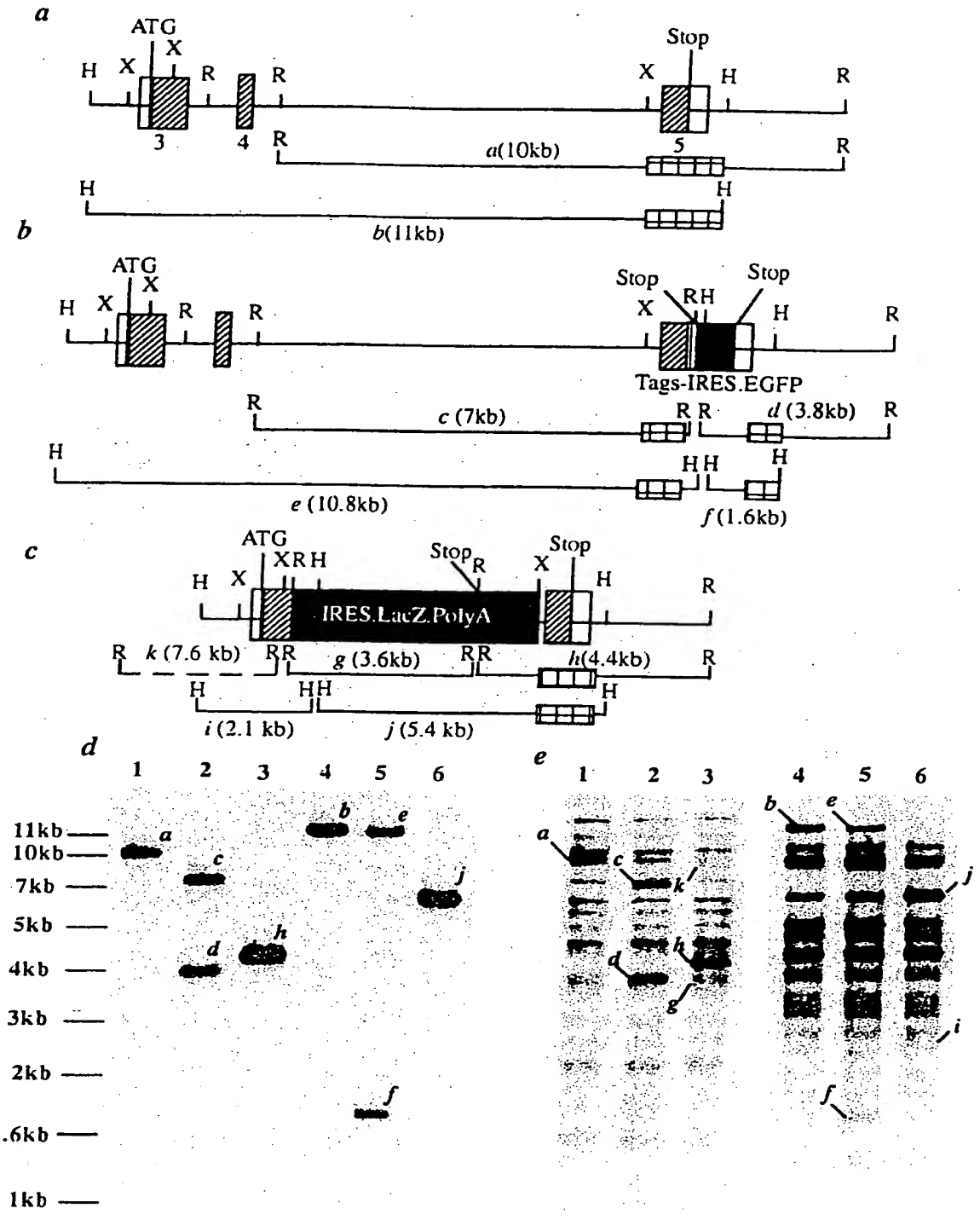

a
ATG
Stop
a(10kb)
b(11kb)
b
Stop
Stop
Tags-IRES.EGFP
c (7kb)
d (3.8kb)
e (10.8kb)
f (1.6kb)
c
IRES.LacZ.PolyA
k (7.6 kb)
g (3.6kb)
h(4.4kb)
i (2.1 kb)
j (5.4 kb)
d
e
11kb
10kb
7kb
5kb
4kb
3kb
2kb
1.6kb
1kb

Figure 14

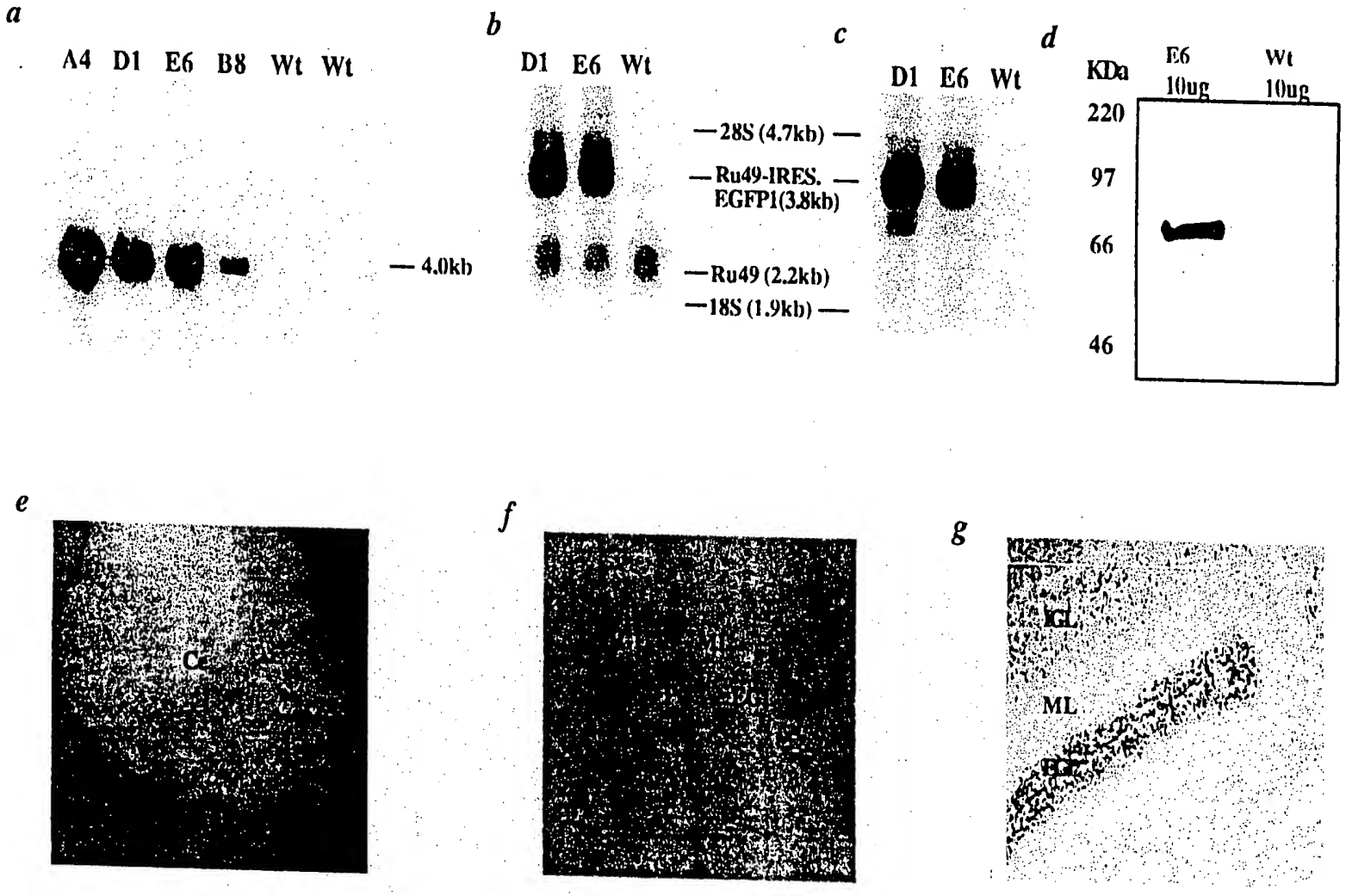

a
A4 D1 E6 B8 Wt Wt
— 4.0kb
b
D1 E6 Wt
c
D1 E6 Wt
—28S (4.7kb) —
— Ru49-IRES. —
EGFP1(3.8kb)
—Ru49 (2.2kb)
—18S (1.9kb) —
d
KDa
E6
10ug
Wt
10ug
220
97
66
46
e
C
f
g
EGL
ML

Figure 15

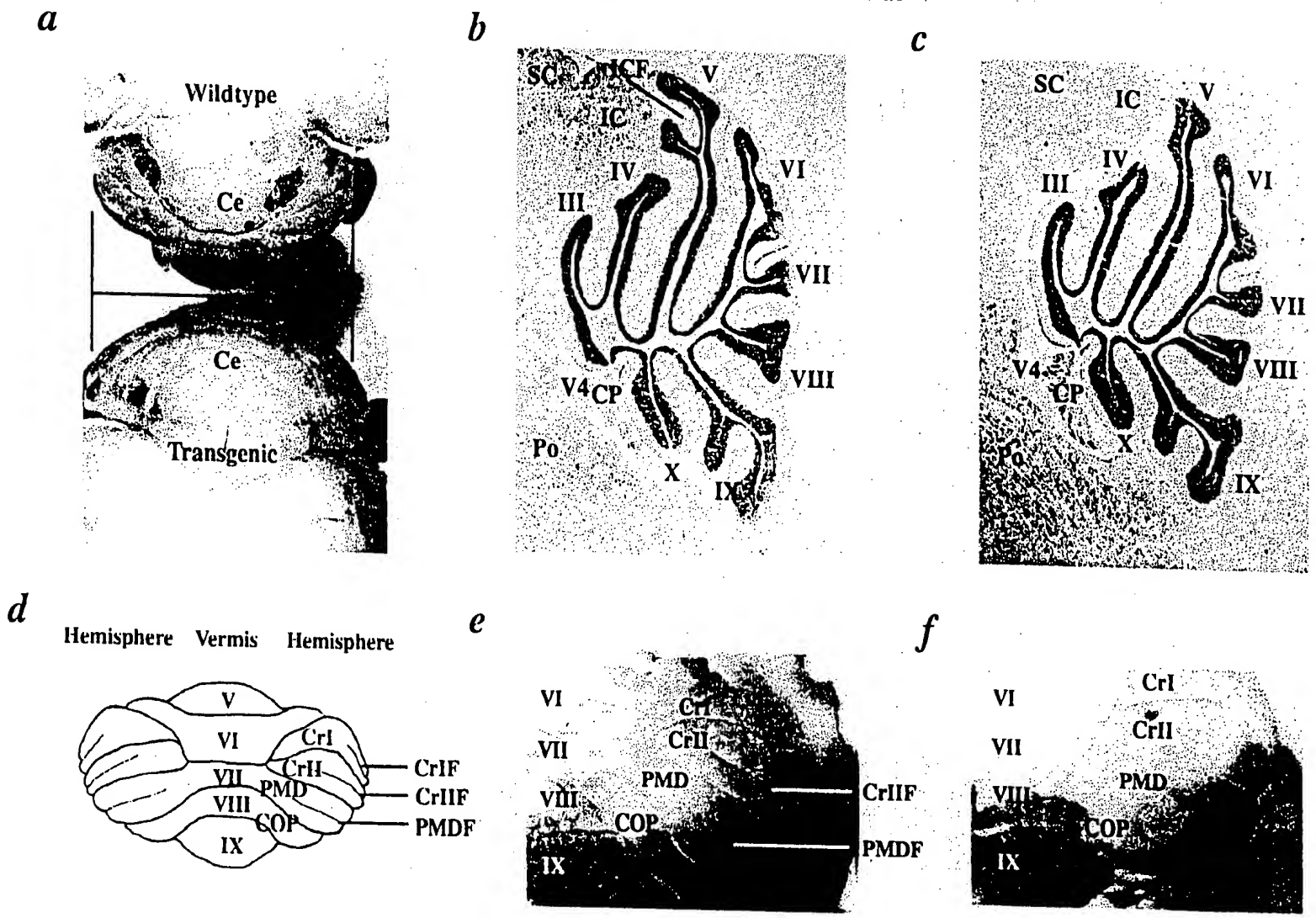

a
Wildtype
Ce
Ce
Transgenic
b
SC
ICF
V
IC
IV
VI
III
VII
VIII
V4
CP
Po
X
IX
c
SC
IC
V
IV
III
VI
VII
V4
CP
VIII
Po
X
IX
d
Hemisphere
Vermis
Hemisphere
V
VI
CrI
CrII
VII
PMD
VIII
COP
IX
CrIF
CrIIF
PMDF
e
VI
CrI
CrII
VII
PMD
VIII
COP
IX
CrIIF
PMDF
f
VI
CrI
CrII
VII
PMD
VIII
COP
IX

Figure 16

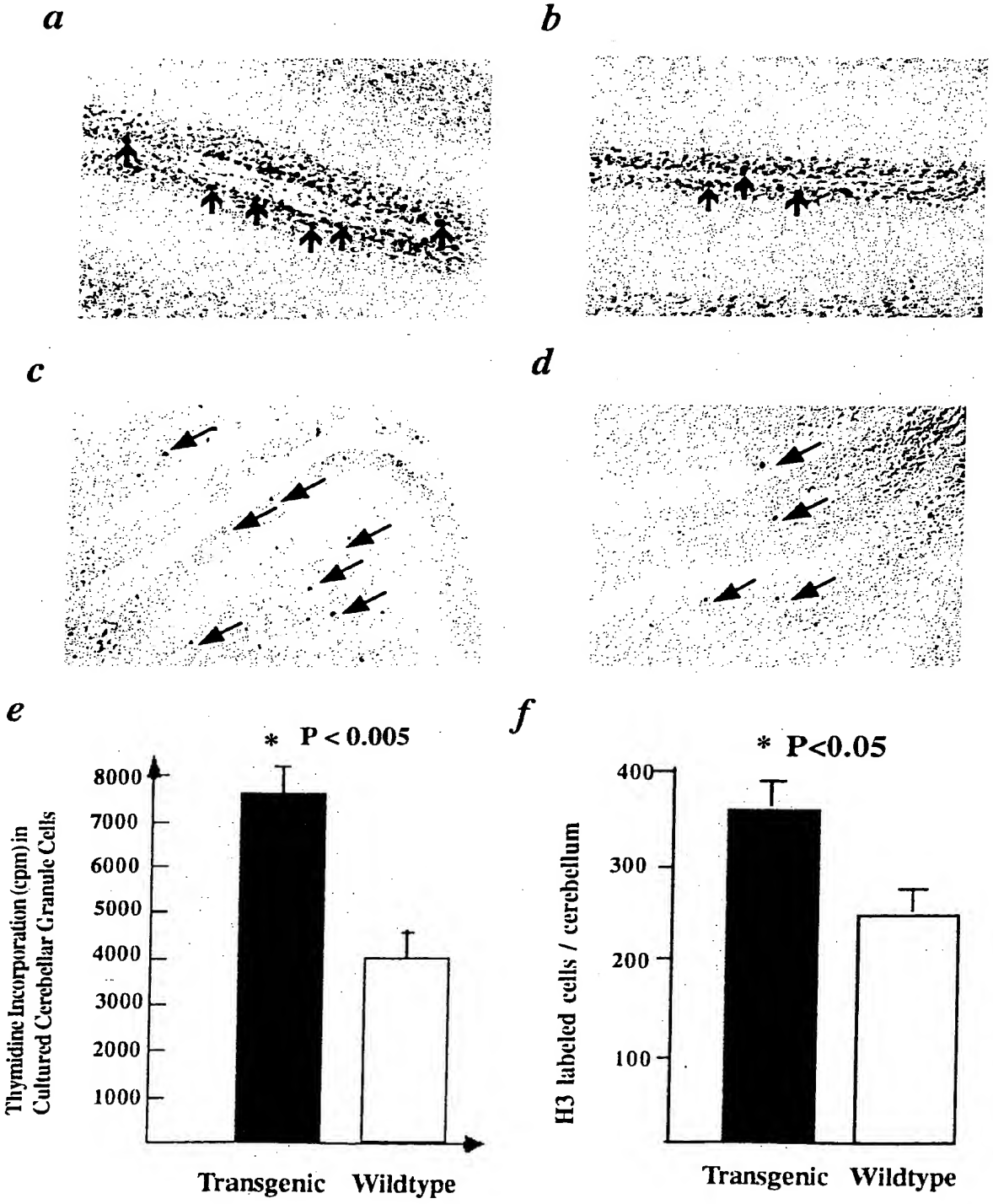

a
b
c
d
e
* P < 0.005
Thymidine Incorporation (cpm) in Cultured Cerebellar Granule Cells
8000
7000
6000
5000
4000
3000
2000
1000
Transgenic
Wildtype
f
* P<0.05
H3 labeled cells / cerebellum
400
300
200
100
Transgenic
Wildtype

Figure 17

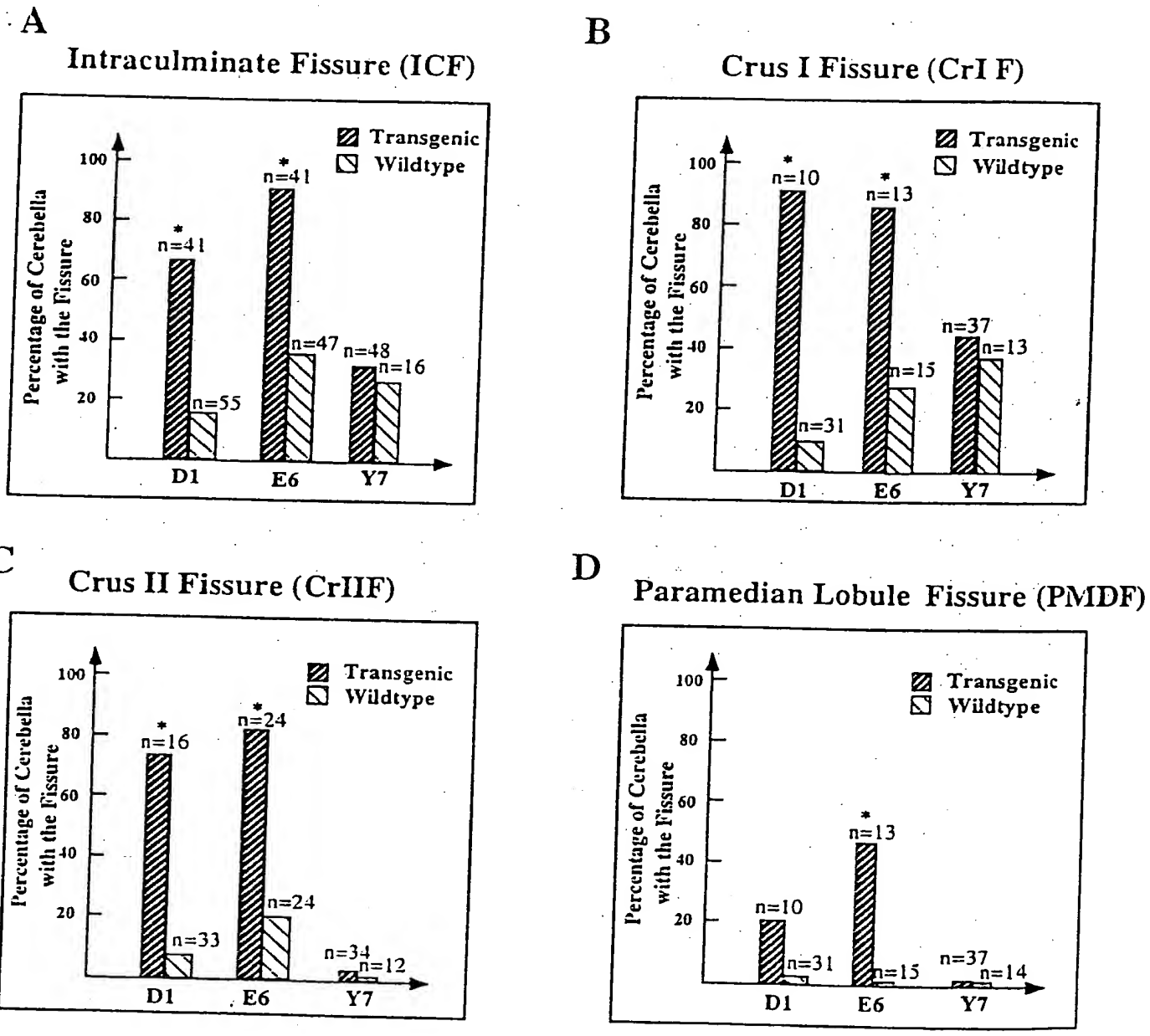

A
Intraculminate Fissure (ICF)
Transgenic
Wildtype
Percentage of Cerebella with the Fissure
100
80
60
40
20
*n=41
n=55
*n=41
n=47
n=48
n=16
D1
E6
Y7
B
Crus I Fissure (CrI F)
Transgenic
Wildtype
Percentage of Cerebella with the Fissure
*n=10
n=31
*n=13
n=15
n=37
n=13
D1
E6
Y7
C
Crus II Fissure (CrIIF)
Transgenic
Wildtype
Percentage of Cerebella with the Fissure
*n=16
n=33
*n=24
n=24
n=34
n=12
D1
E6
Y7
D
Paramedian Lobule Fissure (PMDF)
Transgenic
Wildtype
Percentage of Cerebella with the Fissure
n=10
n=31
*n=13
n=15
n=37
n=14
D1
E6
Y7

Figure 18

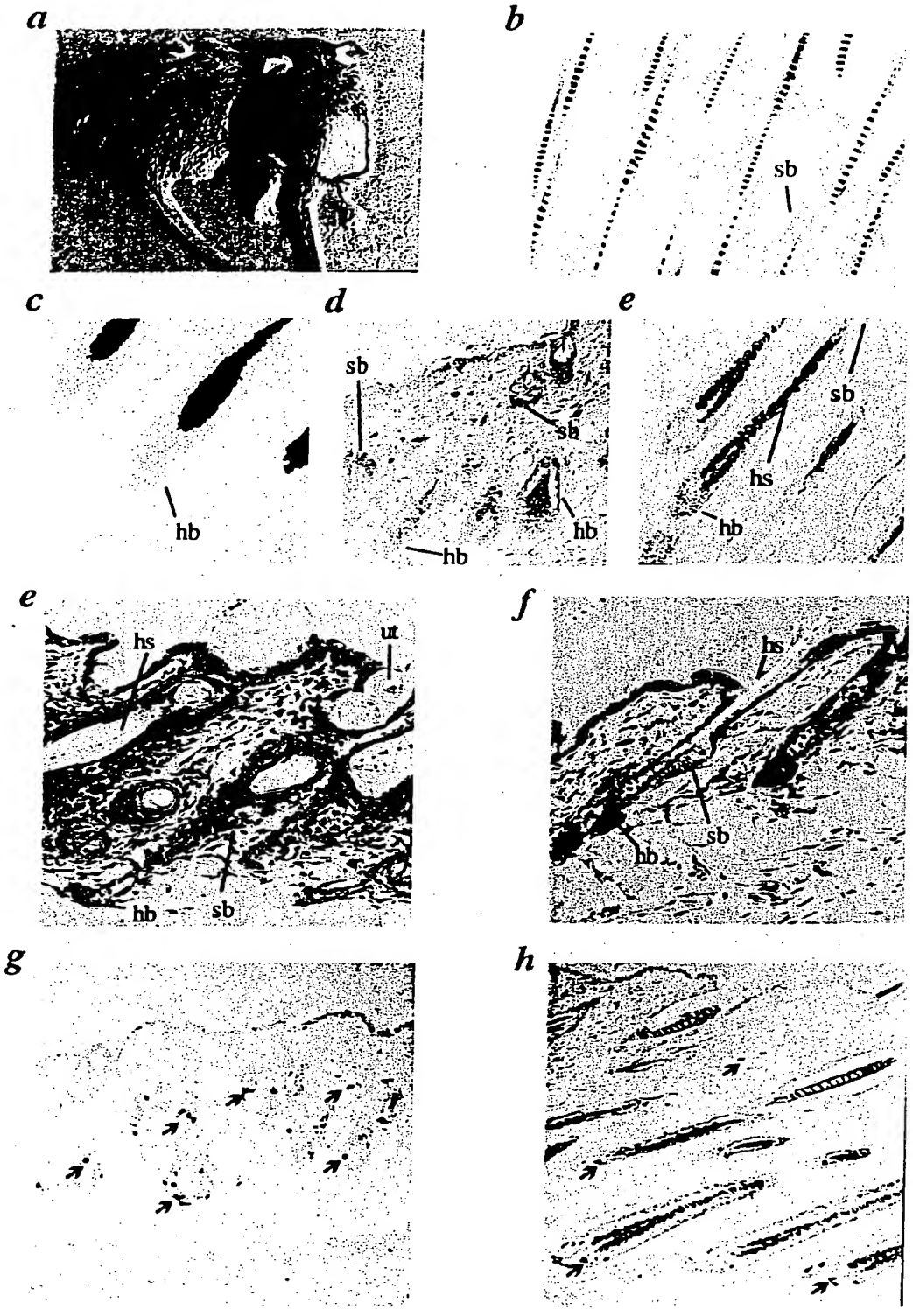


Figure 19

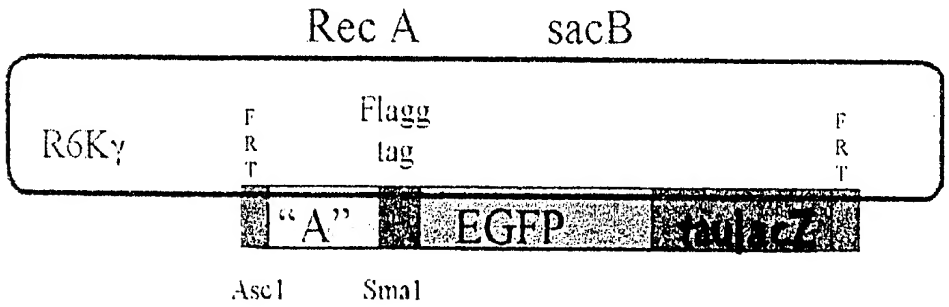


Figure 20

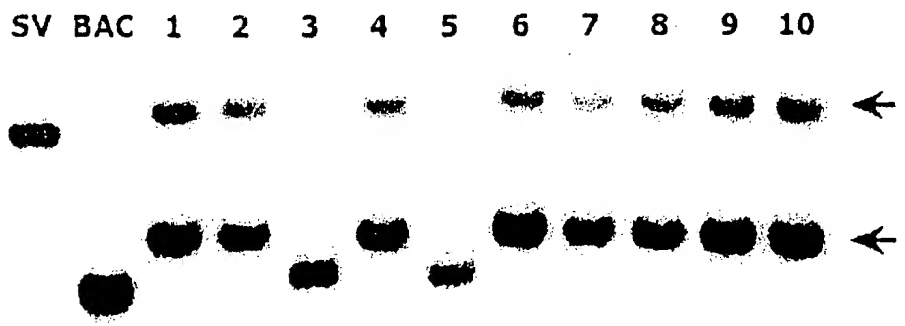


Figure 21

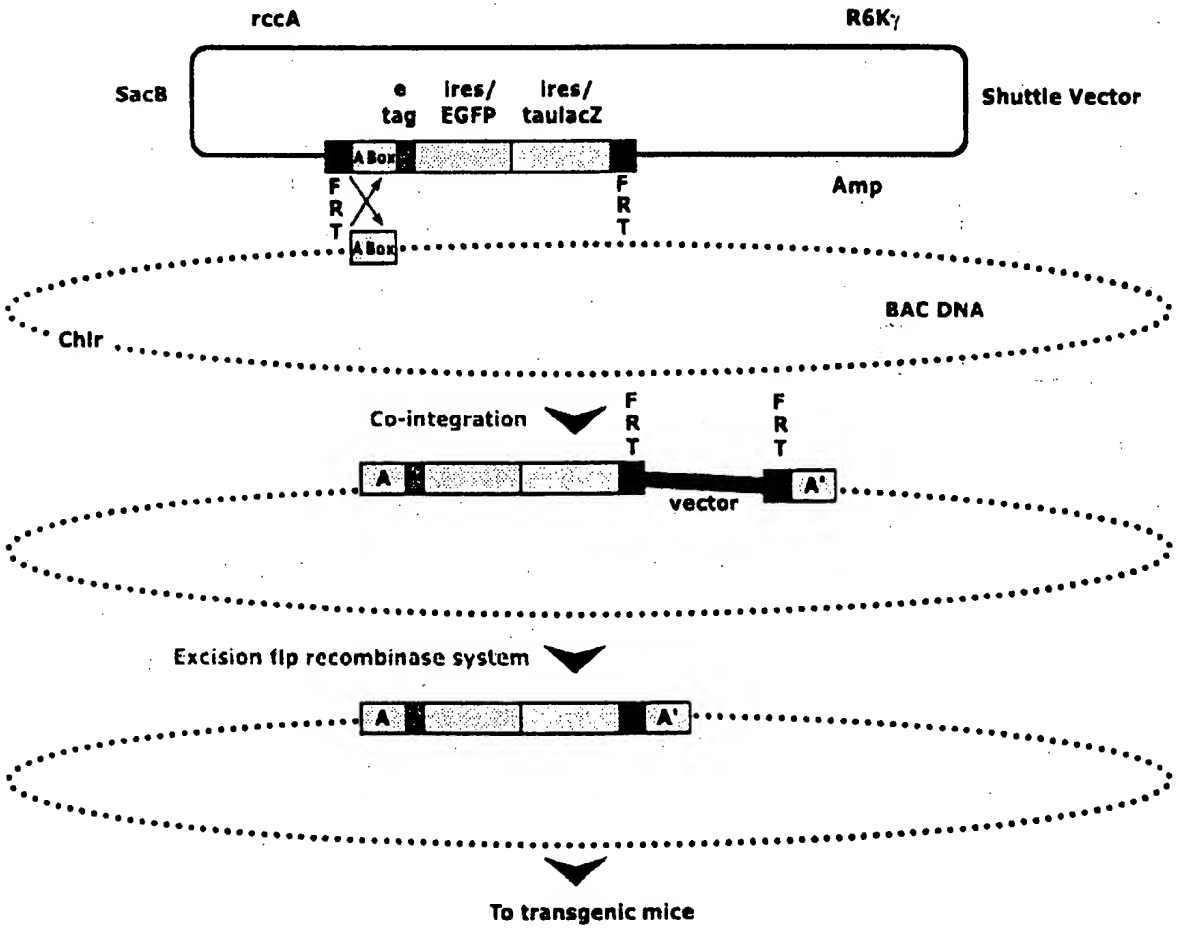

rccA
R6Kγ
SacB
e tag
ires/ EGFP
ires/ taulacZ
Shuttle Vector
A Box
Amp
F R T
F R T
A Box
BAC DNA
ChIr
Co-integration
F R T
F R T
A
A'
vector
Excision flp recombinase system
A
A'
To transgenic mice

Figure 22

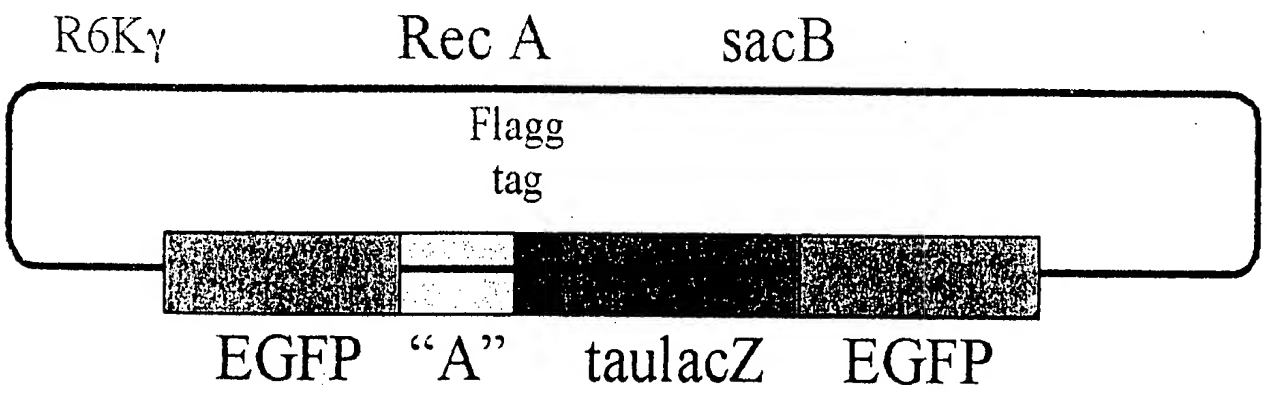


Figure 23

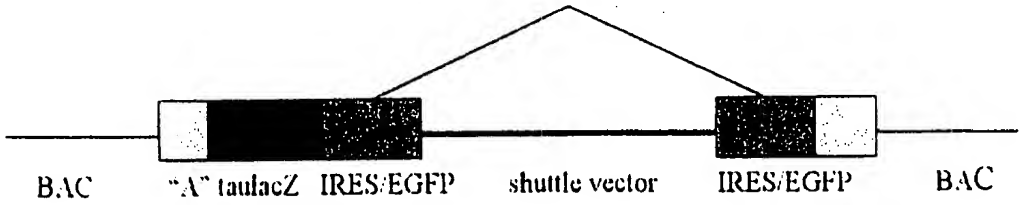


Figure 24

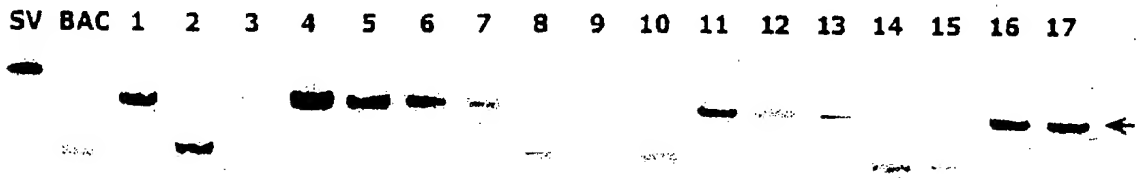


Figure 25